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Systemic recovery and therapeutic effects of transplanted allogenic and xenogenic mesenchymal stromal cells in a rat blunt chest trauma model

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Abstract

Background. Effective therapy of Acute Lung Injury (ALI) is still a major scientific and clinical problem. To define novel therapeutic strategies for sequelae of blunt chest trauma (TxT) like ALI/Acute Respiratory Distress Syndrome, we have investigated the immunomodulatory and regenerative effects of a single dose of *ex vivo* expanded human or rat mesenchymal stromal cells (hMSCs/rMSCs) with or without priming, immediately after the induction of TxT in Wistar rats. *Methods.* We analyzed the histological score of lung injury, the cell count of the broncho alveolar lavage fluid (BAL), the change in local and systemic cytokine level and the recovery of the administered cells 24 h and 5 days post trauma. *Results.* The treatment with hMSCs reduced the injury score 24 h after trauma by at least 50% compared with TxT rats without MSCs. In general, TxT rats treated with hMSCs exhibited a lower level of pro-inflammatory cytokines (interleukin [IL]-1B, IL-6) and chemokines (C-X-C motif chemokine ligand 1 [CXCL1], C-C motif chemokine ligand 2 [CCL2]), but a higher tumor necrosis factor alpha induced protein 6 (TNFAIP6) level in the BAL compared with TxT rats after 24 h. Five days after trauma, cytokine levels and the distribution of inflammatory cells were similar to sham rats. In contrast, the treatment with rMSCs did not reveal such therapeutic effects on the injury score and cytokine levels, except for TNFAIP6 level. *Conclusion.* TxT represents a suitable model to study effects of MSCs as an acute treatment strategy after trauma. However, the source of MSCs has to be carefully considered in the design of future studies.

Key Words: *blunt chest trauma, inflammation, lung contusion, mesenchymal stromal cells, rodents*

Introduction

Severe blunt chesttrauma (TxT) often leads to Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) with a mortality rate of >30% [1,2]. Clinical signs of ARDS are severe hypoxemia, decreased lung compliance, microscopic evidence of alveolar damage and bilateral pulmonary infiltrates [3]. Mechanical ventilation, prone positioning, antimicrobial therapy, neuromuscular blocking agents and conservative fluid management are therapeutic approaches for ALI/ARDS [4]. However, causal therapeutic approaches to improve regeneration or to reduce morbidity and mortality are still missing. Mesenchymal stromal cells (MSCs) as treatment option for ALI have been proposed. Three clinical trials dealing with ALI/ARDS and bone marrow–derived MSC therapy are currently listed on clinicaltrials.gov [5].

MSCs are multipotent cells of peri-vascular origin and are distributed in tissues of the whole body [6]. They have regenerative and immunomodulatory potential by differentiation and secretion of trophic factors [7–10]. Human MSCs are characterized by plastic adherence, at least tri-lineage differentiation, by expression of identity surface markers such as CD73, CD90 and CD105 and by lack of expression of impurity markers

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like CD11b, CD45 and CD14 [11]. Bone marrow and adipose tissue are often used as starting material for MSC expansion because of easy accessibility [12]. Medium supplemented with xenogenic fetal bovine serum has usually been used for expansion of MSCs. However, alternatives for animal serum like platelet lysate have become more popular [13–15]. Of note, the MSCs used in the present animal experiment were isolated, characterized and expanded according to good manufacturing practice (GMP) protocols used in clinical trials for bone regeneration (clinicaltrials.gov, NCT02751125, NCT02065167 and NCT01842477). These cells showed significant therapeutic effects in wound healing and long bone defects in pre-clinical studies [7,8]. Previous studies showed that priming of these MSCs with pro-inflammatory cytokines (e.g., with tumor necrosis factor [TNF] in combination with interferon gamma) resulted in the expression of immunomodulatory factors or to enhanced immunosuppressive effects compared with resting MSCs [8,9]. Priming significantly increases the expression of costimulatory and adhesion molecules (e.g., CD40, CD54, CD106) and immunomodulatory cytokines like indoleamine 2,3-dioxygenase 1, nitric oxide synthase 2, prostaglandin-endoperoxide synthase 2 and TNF alpha induced protein 6 (*TNFAIP6*). Furthermore, the inhibition of T-cell, B-cell and natural killer (NK)-cell proliferation is induced or enhanced by priming. In addition, the susceptibility to NK-mediated lysis is reduced after priming [9]. TNFAIP6 is known as an anti-inflammatory protein and plays an important role in tissue remodeling. Some therapeutic effects of MSCs are explained by TNFAIP6 secretion or increased after *TNFAIP6* overexpression in animal models of myocardial infarction [16], peritonitis [17], wound healing [8] and lipopolysaccharide (LPS)-induced lung injury [18]. *TNFAIP6* can be induced by the pro-inflammatory cytokines interleukin (IL)-1 beta (1B) and TNF [8,17,19,20]. IL-1B stimulation of MSCs is known to increase the synthesis of chemokines, interleukins, Toll-like receptors, metalloproteinases, growth factors and adhesion molecules mainly by activation of the nuclear factor kappa-light-chain-enhancer of activated B-cells signaling pathway [19,21]. Furthermore, IL-1B pre-treatment (10 ng/mL for 48 h) of human UC-MSCs has shown therapeutic benefit in a dextran sulfate sodium-induced colitis model in C57BL/6J mice [22]. This benefit was explained by increased migratory ability and immunosuppressive effects of primed MSCs. In previous work we have investigated the effects of a defined serum-free polytrauma "cocktail" containing IL-1B, IL-6, IL-8 and the anaphylatoxins C3a and C5a in concentrations corresponding to those measured in the blood of polytrauma patients, on human MSCs (hMSCs) *in vitro* [19]. We could demonstrate that IL-1B is a crucial mediator of the polytrauma cocktail in terms of immune-modulation and matrix metallopeptidase 1 (MMP1) expression. Also, IL-1B was found to be increased in the serum of polytrauma patients early after injury [23].

MSCs represent a potential treatment option for ALI/ARDS because of their anti-microbial [24,25], anti-inflammatory [8,10] and pro-angiogenic effects [26]. The effect of MSCs in pre-clinical models for ALI has already been tested for infection- and chemical-induced lung injury in different species [18,27,28]. Furthermore, it was shown that pre-treatment of MSCs had an increased therapeutic benefit in pre-clinical studies of oxygen-induced neonatal lung injury [29], bleomycin-induced lung injury [30] and LPS-induced lung injury [31]. So far there is no clinical study using cytokine pre-treated MSCs for the treatment of lung injury. However, the concept of primed MSCs has been used in other indications (e.g. clinical trials for heart failure used pre-treated MSCs). In the pilot trial "Mesenchymal Stem Cells and Myocardial Ischemia" (MESAMI 1) cell growth of bone marrow derived MSCs was supported by fibroblast growth factor 2 (FGF2) in cell culture medium [32]. In the C-CURE Multicenter Randomized Trial a cytokine cocktail was used to induce cardiogenic differentiation [33].

The present study focuses on the use of bone marrow MSCs in a well-established TxT model in rats. The ensuing sterile inflammation was treated with an intravenous (i.v.) single-dose MSC administration. Pre-stimulated MSCs were additionally tested. We analyzed recovery of i.v.-administered MSCs from blood and broncho alveolar lavage fluid (BAL) fluids of the rats, the characteristics of the recovered MSCs and the effect of MSCs on trauma-induced lung injury.

Methods

Study protocol

Six groups of animals were implemented to determine if administration of xenogenic or allogenic MSCs has an effect on inflammatory and regenerative processes: (i) sham, (ii) traumatized rats (TxT) and rats receiving (iii) hMSCs or (iv) rat MSCs (rMSCs) after TxT (hMSCs/rMSCs). Further groups included IL-1B–primed hMSCs (v) or rMSCs (vi). To recover the injected MSCs and to distinguish between endogenous and the administered MSCs, we performed PKH26 staining. Twenty-four hours or 5 days after TxT, we collected blood, BAL, lungs and other tissues. Flow cytometry analysis was performed to detect and characterize the administered MSCs in blood and BAL. In addition, BAL fluid was analyzed using Luminex and enzyme-linked immunosorbent assays (ELISAs). Paraffin sections of the lungs were scored for

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